Bacterial populations of litter and soil in a deciduous woodland

1. — Qualitative studies

BY

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INTRODUCTION

Work has been initiated at Meathop Wood, Lancashire, England, as part of the International Biological Programme, to study the relative importance of the different groups of soil organisms in the process of decay and transfer of energy and nutrients within the soil (SATCHELL, 1970; GRAY and WILLIAMS, 1971). Information is being obtained on the amount of energy and nutrient yielding material entering the plant-soil ecosystem, the types and biomasses of the organisms present and the partitioning of energy and nutrients between them. Amongst the important groups of soil organisms present are the aerobic heterotrophic bacteria and in this paper information is presented on the range of these organisms found in the litter and soil. A phenetic classification of the isolated organisms has been produced, enabling keys to be constructed for the identification of the commonest types of bacteria. The procedure used was essentially the same as that described by GOODFELLOW (1968) and GOODFELLOW, HILL and GRAY (1968) and LOWE and GRAY (1973) in their studies on the bacteria of coniferous woodland soils.

MATERIALS AND METHODS

Site: Meathop Wood is situated at the head of Morecambe Bay, Lancashire National Grid Reference SD (34)435795, on an outcrop of Carboniferous

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limestone, with terraces and small scarps, between 30 and 55 metres above sea level. A layer of glacial drift covers the limestone.

The soil is predominantly a brown earth, varying greatly in depth from 0-100 cm with an average depth of 39 cm. The soil profile is ill-defined with mull humus, a mid-brown A_1 horizon and a lighter orange C horizon. Because of high earthworm activity, much of the surface leaf litter is removed at an early stage of decomposition and so the humus layer is very thin. The reaction of the soil fluctuates randomly, although it is highest where the soil is shallow: values between 4.5 and 6.0 are common but values of 4.2 and 7.3 do occur. The moisture tension at 5-9 cm depth is below pF 2.3 throughout most of the year and at times the soil may become saturated with water. Over a seven year period, the annual rainfall varied from 97.5 - 145.9 cm, while soil temperature varied from 1.6° (February) to 13.9° (July/August) at the surface and from 2.2° (February) to 13.5° (August) at a depth of 10 cm during 1967 and 1968.

The dominant trees were *Quercus petraea, Q. petraea* x *robur, Fraxinus excelsior, Betula pubescens* x *verrucosa* and *Acer pseudoplatanus* with a well developed understory of *Corylus avellana.* The ground flora was rich and varied and in different parts of the site, the dominant species were *Mercurialis perennis, Rubus fruticosus* and *Oxalis acetosella. Endymion non-scripta* was also common. The annual litter production from these sources is summarized in Table 1.

TABLE 1

Annual litter production at Meathop Wood (average over five years). (excluding roots) hibberd (pers. comm.)

Source of litter	Amount of annual litter production (kg per ha)
Oak leaves	1 408.8
Ash leaves	771.0
Birch leaves	372.9
Hazel leaves	498.8
Sycamore leaves	102.9
Other tree leaves	91.3
TOTAL	3 245.7
Twigs and branches	1 759.7
Fruits	153.9
Other tree material	446.6
TOTAL	2 360.2
Ground flora	689.7
Organic matter in rainfall	415.9
GRAND TOTAL	6 711.5

The great variability of soil and vegetation make this soil difficult to study from a microbiological point of view.

Isolation of bacteria: Bacteria were isolated from four separate environments within the woodland, i.e. decaying oak leaves, decaying ash leaves, the humus layer and the mineral soil at 5.0 cm. depth. Dilution plates were prepared from litter samples by first macerating the litter in 200 ml quarter-strength Ringer's solution at half speed for 7 mins. in an MSE Atomix blender. Ten-fold dilutions were prepared from this material and plated on peptone yeast extract agar with added actidione (GOODFELLOW, HILL and GRAY, 1968) and nystatin (WILLIAMS and DAVIES, 1965). Similar plates were prepared from the humus and mineral soil samples but these samples were dispersed initially by magnetic stirring (HILL and GRAY, 1966). Colonies which had grown after 14 days incubation at 25° were selected at random, purified and stored on nutrient agar slopes under oil. Dilution plates were prepared at monthly intervals from November to June and bacteria isolated until 100 strains had been acquired from each habitat.

Characterization of isolates: All isolates were examined for a variety of morphological and physiological characteristics. These tests are listed in Table 2. Many of the physiological tests were carried out using multipoint inoculation methods (GOODFELLOW and GRAY, 1966, SNEATH and STEVENS, 1967). Unless indicated, all cultures were incubated for 1 week at 25°. The results of these tests were recorded as positive or negative and transferred onto punch cards in the form of 131 separate characters.

TABLE 2

Characters determined for each organism

Cell length, less than 0.8 μm, 0.8 μm - 1.4 μm, 1.5 - 2.5 μm, over 2.5 μm.
Cell width, less than 0.5 μm, 0.5 - 0.9 μm, over 0.9 μm.
Motility.
Production of irregularly shaped cells.
Arrangement of cells in chains, clumps, etc.
Production of endospores on nutrient or soil extract agar.
Position of endospore in sporangium.
Shape of sporangium.
Uptake of malachite green by cells.
Gram positive, variable or negative.
Production of extracellular slime.
Shape of colony.
Nature of colony margin.
Nature of colony elevation.
Colour of pigment.
Diffusibility of pigment.
Fluorescence of pigment.
Production and type of surface growth in nutrient broth.
Growth at pH 4.0, 5.0, 6.0, 8.0, 9.0, 10.0.
Growth at 10°, 15°, 30°, 35°, 40°, 45°.
Use of ammonium, aspartic acid or cysteine as sole nitrogen sources.
Growth in 3 %, 5 %, 7 %, 10 % and 15 % NaCl.
Growth in 0.1 % phenol, 0.01 % sodium azide and 0.05 % potassium tellurite.
Growth with 0.01 % or 0.001 % crystal violet.
Resistance to chlortetracycline, chloramphenicol, bacitracin, penicillin G, erythromycin, streptomycin, polymyxin B, novobiocin.

Table 2 cont.

Production of catalase.
Oxidative or fermentative attack of glucose in 24 hr, 1 week and 2 weeks.
Production of gas from glucose.
Production of alkali in Hugh and Leifson's medium.
Acid from L-arabinose, D.-arabinose, xylose, fructose, galactose, rhamnose, mannose, glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melizitose, raffinose, glycogen, inulin, amygdalin; salicin, dulcitol, glycerol, mannitol, sorbitol, inositol.
Hydrolysis of aesculin, gelatin, casein, tributyrin, xylan, chitin, laminarin, pectin, starch.
Production of acetoin from glucose.
Production of oxidase, urease and arginine decarboxylase.
Reduction of nitrate to nitrite and nitrogen.
Utilization of sole carbon sources, citrate, acetate, gluconate, lactate, succinate, tartrate, oxalate, glucosamine, p-hydroxybenzoic acid, vanillic acid, alginate.

Computation of results: The organisms were compared with one another and similarity and matching coefficients were calculated for each pair of organisms (SNEATH, 1957; SOKAL and MICHENER, 1958). From these results, dendrograms, similarity matrices and minimum spanning trees were produced, using the Orion computer at Rothamsted (LOWE and GRAY, 1972).

RESULTS

Clustering of the isolates.

The results of the cluster analysis showed that substantially the same clusters of isolates were obtained using similarity or matching coefficients. The principal difference was that the matching coefficient enabled groups to be defined at the 80 % level of similarity while the similarity coefficient gave clusters defined at the 60-70 % level. Only the results obtained using the similarity coefficient are described here.

Fig. 1 is a minimum spanning tree, showing the clustering of isolates, in which the relationships of the different groups to one another can be seen, as well as the habitats from which they were isolated.

The characteristics properties and approximate taxonomic position of the groups defined are set out below.

Group 1.

Gram negative, non-sporing rods, unable to grow on simple carbon or nitrogen sources. Unable to hydrolyse gelatin, casein, chitin, laminarin or starch, but otherwise variable in their properties.

Group 1a.

Gram negative, non-sporing rods, generally inactive in the tests used. Acid rarely produced from sugars and proteins and polysaccharides not attacked. Possibly *Achromobacter.*

Group 2a.

Gram positive, non-sporing rods, unable to grow in simple carbon or nitrogen sources. Acid produced oxidatively from glucose and several other sugars. Nitrate not reduced. *Arthrobacter.*

Group 2b.

Gram positive, non-sporing rods, unable to grow on simple carbon or nitrogen sources. Acid produced oxidatively from glucose and a few others sugars. Nitrate not reduced. *Arthrobacter.*

Group 3.

Gram positive or Gram variable, non-sporing rods, unable to grow on simple carbon or nitrogen sources. Acid from xylose but otherwise inactive in the tests used. *Arthrobacter.*

Group 4a.

Large, Gram positive, spore-forming rods. Able to use gluconate as a sole carbon source and hydrolyse gelatin, casein, tributyrin and laminarin. *Bacillus cereus- megatherium* intermediates.

Group $4b_1$.

Large, Gram positive, spore forming rods, unable to use simple carbon or nitrogen sources. Produces acid from glucose fermentatively, as well as a few other sugars. Hydrolyses a variety of proteins and polysaccharides and reduces nitrate to nitrite. *Bacillus cereus* and *B. cereus mycoides.*

Group $4b_2$.

Gram positive and Gram variable, spore forming rods. Unable to use simple carbon and nitrogen sources. Produces acid from glucose fermentatively as well as a few other sugars. Hydrolyses gelatin and some polysaccharides but does not attack casein. Reduces nitrate to nitrite. *B. circulans/laterosporus/brevis* complex.

Group 4c.

Gram positive, spore forming rods, generally biochemically inactive. Probably a mixture of several closely related *Bacillus* species.

Group 4d.

Gram variable, spore forming rods. Able to use gluconate and lactate as sole carbon sources. Produces acid from glucose fermentatively, as well as many other sugars. Grows at 45° and in the presence of 10 % sodium chloride. Produces acetoin. *Bacillus subtilis.*

Group $4e_1$.

Gram variable, spore forming rods. Unable to use simple carbon and nitrogen sources. Produces acid weakly from glucose and some other sugars. Unable to hydrolyse gelatin or casein but attacks xylan, laminarin and starch. Probably *Bacillus circulans.*

Group $4e_2$.

Gram negative, spore forming rods. Unable to use simple carbon and nitrogen sources. No growth at 35° or in presence of 3 % sodium chloride. Casein not hydrolysed but gelatin and laminarin are. Little or no acid production from sugars. *Bacillus circulans* variant.

Group 6.

Gram positive, non-motile, non-spore forming rods with rudimentary branching. No growth at 35°. Simple nitrogen sources not utilised and growth on simple carbon sources poor. Does not produce acid from sugars or hydrolyse proteins and polysaccharides. *Mycobacterium.*

Group 7a.

Gram negative, non-sporing motile rods. Tolerate 5 % sodium chloride and 0.01 % crystal violet. Citrate and gluconate used as sole carbon sources. No hydrolysis of proteins or polysaccharides. Produces acid from glucose and a few other sugars oxidatively. Arginine decarboxylase not produced. *Pseudomonas* Group II (SHEWAN *et al.*, 1960).

Group 7b.

Gram-negative, non-sporing, motile rods. Tolerate 0.01 % crystal violet and several antibiotics. Utilize citrate, gluconate and lactate as sole carbon sources. Produces yellow-green fluorescent pigment on gluconate. Produces acid from glucose and several other sugars oxidatively. Produces arginine decarboxylase but not acetoin. *Pseudomonas* Group I (SHEWAN *et al.*, 1960).

Group 7c.

Gram negative, non-sporing, motile rods. Tolerate 0.01 % crystal violet and several antibiotics including 10 µg chlortetracycline. Utilize gluconate, lactate and succinate as sole carbon sources. Acid from glucose and a few other sugars oxidatively. Do not attack proteins or polysaccharides. No pigment produced. *Pseudomonas* Group II (SHEWAN *et al.*, 1960).

Group 7d_1.

Gram negative, non-sporing rods. Tolerate 7 % sodium chloride, 0.01 % crystal violet and 0.1 % phenol. Utilize citrate, gluconate, lactate and succinate as sole carbon sources and ammonium, aspartate and cysteine as sole nitrogen sources. Produce acid from glucose and many other sugars fermentatively. No action on proteins or polysaccharides. Paracolon group, e.g. *Escherichia, Paracolobactrum.*

Group 7d_2.

Gram negative, non-sporing rods. Tolerate 7 % sodium chloride, 0.01 % crystal violet and 0.1 % phenol. Utilize citrate, gluconate, lactate and glucosamine as sole carbon sources. Acid from glucose and several other sugars fermentatively. No acid from sucrose, raffinose and mannitol. Inactive against proteins and polysaccharides. *Coli-aerogenes* group, e.g. *Escherichia, Klebsiella.*

Group 8.

Gram positive cocci, unable to use simple carbon and nitrogen sources. Produce acid from glucose and a few other sugars fermentatively. Otherwise biochemically inactive. *Staphylococcus.*

Using the above characteristics and a few others, a key has been produced to these organisms (HISSETT, 1970).

Distribution of the clusters.

The above clusters showed some striking distribution patterns in the four soil and litter habitats. Table 3 shows that with the exception of Group 8 *(Staphylococcus)*, the organisms were associated primarily with one habitat.

TABLE 3

Distribution of the main clusters of bacteria in different soil environments

Group	Percentage of isolates				
	Ash litter	Oak litter	Humus layer	Soil (A_1)	Total
1. G-ve rods	5	—	5	2	2.9
1a. *Achromobacter*	—	—	11	—	2.5
2a. *Arthrobacter*	18	4	—	—	5.1
2b. *Arthrobacter*	10	2	—	—	2.8
3. *Arthrobacter*	17	—	—	—	3.7
4a. *Bacillus megaterium/cereus*	—	2	4	18	6.1
$4b_1$. *Bacillus cereus/mycoides*	—	—	4	10	3.7
$4b_2$. *Bacillus circulans/laterosporus/brevis*	—	—	2	6	2.2
4c. *Bacillus* spp	—	—	2	10	3.4
4d. *Bacillus subtilis*	5	—	1	—	1.4
$4e_1$. *Bacillus circulans*	1	—	—	7	2.2
$4e_2$. *Bacillus circulans*	—	—	—	5	1.4
6. *Mycobacterium*	—	—	7	1	2.0
7a. *Pseudomonas* Gp. II	18	—	—	—	3.9
7b. *Pseudomonas* Gp. I	5	37	2	—	15.0
7c. *Pseudomonas* Gp. II	—	—	5	3	2.2
$7d_1$. Paracolon group	3	6	—	—	2.2
$7d_2$. *Coli-aerogenes*	—	15	—	—	4.2
8. *Staphylococcus*	3	2	1	1	1.7

On ash litter, the bacterial flora was dominated numerically by Group 2a *(Arthrobacter)*, Group 3 *(Arthrobacter)* and Group 7a (*Pseudomonas* group II). In contrast, oak litter was colonised mainly by Group 7b (*Pseudomonas* group I) and Group $7d_2$ (*coli-aerogenes* organisms).

In the humus layer, Group 1a *(Achromobacter)* and Group 6 *(Mycobacterium)* were the commonest isolates, though many other groups were also present in smaller quantities. In the mineral soil, Group 4a *(Bacillus megaterium/cereus)* predominated together with other *Bacillus* species.

Unclassified strains were found in all habitats, the smallest numbers being from ash litter (15 %) and oak litter (22 %). In the A_1 horizon soil, 27 % of the isolates were unclassified and in the humus layer, 56 % of the isolates were not linked to any clearly defined groups. The heterogeneity of the humus layer isolates was confirmed by analysing them separately and the results of this analysis (HISSETT, 1970) shows the relationship of these unclassified strains with the clearly defined groups. This heterogeneity probably reflects the enormous diversity of leafy and woody materials contributing to the formation of humus.

Distribution of physiological and biochemical properties.

Growth conditions (Table 4). Isolates from litter showed a greater tolerance of sodium chloride than those from the two soil horizons and were

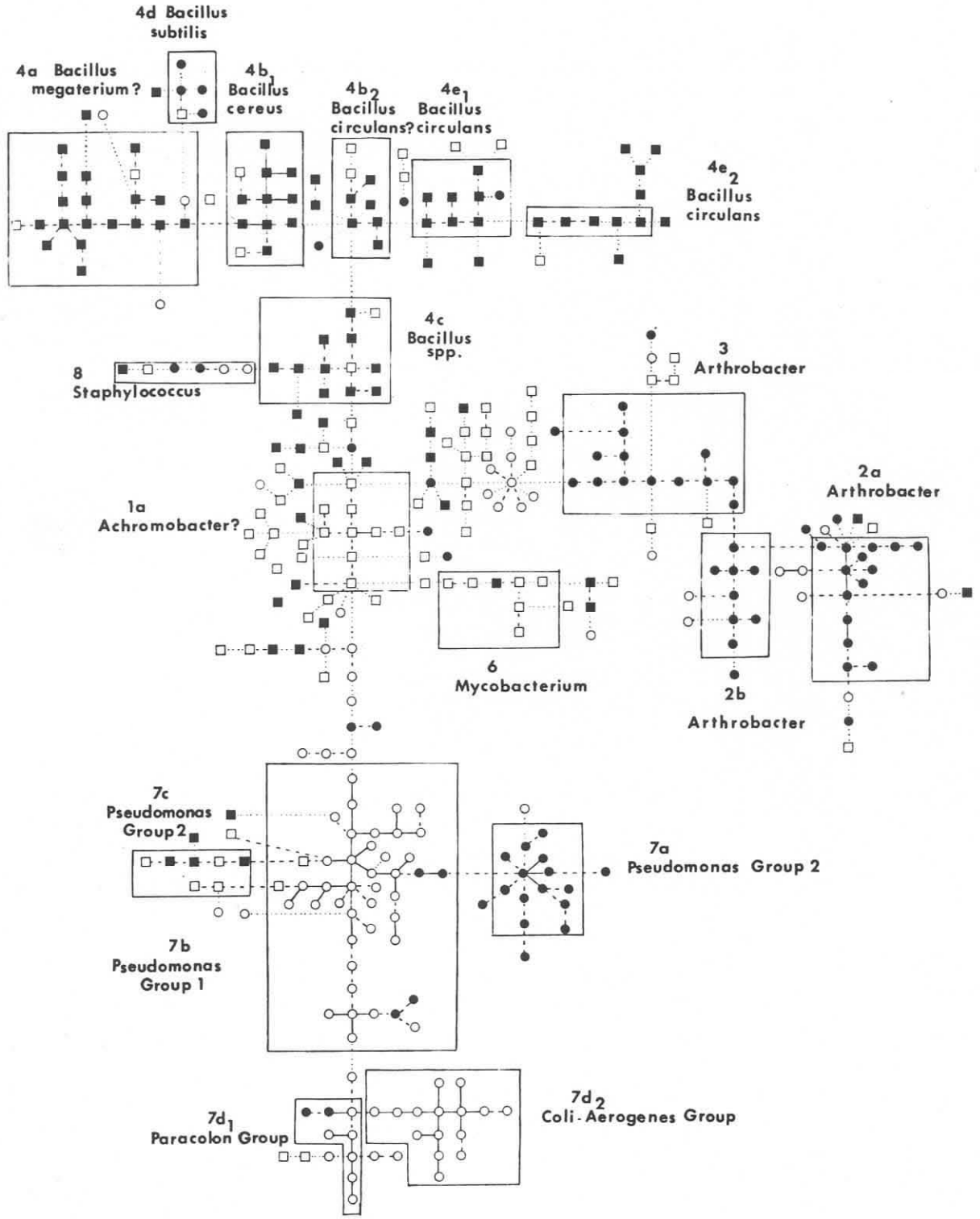

FIG. 1. — Minimum spanning tree showing the clusters of soil, humus and leaf litter strains using a similarity coefficient.

● = strain from ash litter
○ = strain from oak litter
□ = strain from humus layer
■ = strain from mineral soil
——— = 85 % similarity and above
- - - - - = 70-84 % similarity
. . . . . = below 70 % similarity.

Solid lines surrounding groups of strains indicate that these strains form a cluster on the matrix diagrams (HISSETT, 1970), generally at the 50-60 % level.

TABLE 4

Percentage of isolates utilising various sole carbon and nitrogen sources in deciduous and coniferous forest soils

Sole carbon or nitrogen source	Percentage of isolates in different environments					
	Deciduous woodland				Coniferous woodland*	
	Ash litter	Oak litter	Humus layer	Mineral soil (A_1)	Mineral soil (A_1)	Mineral soil (C)
Ammonium	6	38	0	1	50	35
Glucosamine	3	36	5	4	31	35
Acetate	0	1	1	0	15	9
Citrate	27	66	7	10	39	30
Gluconate	32	67	23	23	50	53
Lactate	13	63	14	18	38	38
Oxalate	0	0	2	2	9	8
Succinate	8	54	11	10	32	40
Tartrate	5	6	4	4	8	16
p-hydroxybenzoic acid	0	28	5	4	1	2
Vanillic acid	0	1	6	2	0	2

* GOODFELLOW (1969).

TABLE 5

Percentage of isolates growing under various conditions of incubation from deciduous and coniferous forest soils

Growth condition	Percentage of isolates in different environments					
	Deciduous woodland				Coniferous woodland*	
	Ash litter	Oak litter	Humus layer	Mineral soil (A_1)	Mineral soil (A_1)	Mineral soil (C)
pH 5.0	18	39	17	37	48	31
Temp. 30°	83	83	60	85	96	71
35°	31	18	26	58	80	54
40°	11	6	19	39	68	38
45°	5	0	1	0	50	23
3 % NaCl	96	84	42	64	62	75
5 % NaCl	80	79	20	36	50	66
7 % NaCl	39	34	7	7	30	43
0.1 % phenol	45	55	24	56	43	8
Chloramphenicol	36	49	27	8	3	10
Erythromycin	41	66	23	12	12	12

* GOODFELLOW (1969).

generally resistant to more antibiotics. Over 70 % of oak isolates tolerated 0.01 % crystal violet, compared with less than one third for any of the other habitats.

Sole carbon and nitrogen sources (Table 5). Oak litter isolates showed the simplest nutrient requirements. Very few of the humus or mineral soil isolates could use ammonia or aspartate as a sole nitrogen source and none utilised cysteine.

Utilization of sugars (Table 6). Most isolates produced acid from glucose except in the humus layer, but only a few, all from the litter, produced gas. Most litter isolates attacked glucose oxidatively, while the humus and soil yielded about equal numbers of oxidative and fermentative isolates. Lit-

TABLE 6

Percentage of isolates producing acid from sugars from deciduous and coniferous forest soils

Acid produced from	Percentage of isolates in different environments					
	Deciduous woodland				Coniferous woodland*	
	Ash litter	Oak litter	Humus layer	Mineral soil (A_1)	Mineral soil (A_1)	Mineral soil (C)
Glucose (aerobic)	72	63	29	31	45	41
(anaerobic)	19	31	23	39	38	21
D-arabinose	26	38	6	11	40	12
L-arabinose	55	75	19	14	29	38
D-xylose	76	69	30	27	41	32
D-fructose	28	24	8	23	52	44
D-galactose	65	73	24	19	39	30
D-rhamnose	44	33	10	9	25	23
D-mannose	69	73	24	19	39	30
Cellobiose	36	45	18	43	60	40
D-lactose	20	30	7	19	19	9
Maltose	18	22	14	26	50	45
Sucrose	28	24	16	22	46	42
Trehalose	41	37	16	22	46	38
Glycogen	5	0	11	22	43	8
Inulin	3	1	1	1	13	30
D-melizitose	27	11	6	10	24	22
D-raffinose	29	14	4	5	39	30
Salicin	19	37	17	27	28	26
Amygdalin	35	20	13	15	41	36
Dulcitol	3	6	2	0	22	10
Glycerol	53	58	20	44	50	38
D-mannitol	41	37	10	25	45	40
D-sorbitol	24	24	4	3	47	35
Inositol	41	44	10	18	40	30

* GOODFELLOW (1969).

ter isolates showed the greatest activity on the range of carbohydrates tested and humus isolates the least. The most commonly used sugars were glucose, l-arabinose, xylose, galactose, mannose, glucosamine and glycerol.

Other biochemical properties (Table 7). Mineral soil isolates were most active in hydrolysing complex carbohydrates, e.g. xylan, laminarin, chitin, glycogen and starch, while isolates from the two litter types were least active. Very few strains attacked pectin, but most hydrolysed tributyrin. Proteolytic activity was common in all environments. Most strains were catalase positive and oxidase negative. Ability to reduce nitrate was common in all environments but only a few isolates, mainly from soil, could reduce it to nitrogen gas.

TABLE 7

Percentage of isolates able to carry out miscellaneous biochemical reactions from deciduous and coniferous forest soils

Reaction	Percentage of isolates in different environments					
	Deciduous woodland				Coniferous woodland*	
	Ash litter	Oak litter	Humus layer	Mineral soil (A_1)	Mineral soil (A_1)	Mineral soil (C)
Hydrolyse aesculin	90	55	47	56	55	42
gelatin	42	49	31	56	75	25
casein	32	31	14	34	65	30
tributyrin	87	69	85	83	88	38
xylan	6	5	24	52	24	8
chitin	0	2	2	7	7	5
laminarin	29	20	35	62	2	2
Produce acetoin	11	13	4	9	33	12
catalase	92	93	70	85	100	100
oxidase	11	34	17	14	95	30
urease	15	42	17	22	0	5
Hydrolyse pectin	1	0	0	2	2	8
starch	37	19	28	55	48	30
Nitrate reduced to nitrite	35	36	41	50	74	30

* GOODFELLOW (1969).

DISCUSSION

There have been comparatively few studies carried out on the bacterial flora of decomposing leaf litter and little is known about the species composition of this flora. From this survey, it is clear that the bacterial flora of litters at one site may differ from leaf type to leaf type and that in turn, the flora of the leaf litter is distinct from that of the underlying soil. MARTEN and POHLMAN (1942) showed that most litter bacteria were Gram negative rods, probably belonging to the genera *Achromobacter* and *Flavobacterium* although

Bacillus species were present. *Coli-aerogenes* bacteria have also been reported from leaf surfaces, especially the phyllosphere (RUINEN, 1961), although FRASER *et al.* (1956) have suggested that they arise from grazing animals and pollinating insects. In the present study, they were only found on oak leaves at the beginning of the decomposition period, so the possibility remains that they had been present on the living leaves or else were acquired during the storage of the dry leaves. Their rapid disappearance suggests they are not well adapted as litter colonisers.

Rather more is known about the organisms colonising mineral soils and in common with other authors, *Bacillus* species appear to be numerically important (EGOROVA et al., 1964; HOLDING et al., 1965; GOODFELLOW, 1968). However, some of the species concerned differ. Mishustin and Mirsoeva (1968) suggest that *B. virgulus (circulans ?)* is characteristic of forest podzol soils. GOODFELLOW, HILL and GRAY (1968) and GOODFELLOW (1968) also found *B. circulans* in a partially podsolised pine forest soil but *B. subtilis* was present in even greater numbers. In beech forest soils, Jensen (1963) has reported the occurrence of *B. cereus* as the most common *Bacillus* species but with *B. sphaericus* and *B. circulans* also present. These results are most closely comparable with the present study in which *B. cereus* and *B. circulans* were the commonest forms.

JENSEN (1963) also found a high proportion of Gram-negative bacteria in beech forest soils but he isolated his organisms on a mannitol asparagine salts agar which may be selective for these forms; in this study, the Gram negative group 3 bacteria grew more readily on simple carbon and nitrogen sources than most other organisms.

It is also possible to compare the physiological properties of the organisms isolated from the Meathop Wood soil with those isolated by GOODFELLOW (1969) from a pine forest soil, since many of the tests performed were identical. Unfortunately, no comparison can be made with pine litter which has not been examined. The most instructive comparison can be made between the results of studying isolates from the A_1 horizon in both soils (Table 5, 6 and 7). In general, fewer isolates from the deciduous woodland clay soil were able to attack a range of sugars and sugar alcohols. Fewer were able to utilise ammonia as a nitrogen source or to use a range of simple carbon sources. Their ability to grow under different conditions of pH, temperature, salinity, etc. was similar to those in the pine forest soil. In general, this may be summarized by saying that the deciduous forest soil isolates had a poorer biochemical potential than the pine forest soil isolates.

SUMMARY

The principle types of bacteria in litter and soil from a mixed deciduous woodland soil have been classified using numerical taxonomic procedures. In ash litter, *Arthrobacter* and *Pseudomonas* (group 2) were the numerically dominant forms, whereas in oak litter, *Pseudomonas* (group 1) and *coli-aerogenes organisms* were the most characteristic. In the amorphous humus, *Achromobacter* and *Mycobacterium* were the commonest isolates, while *Bacillus* species were most abundant in the soil. The number of unclassified forms also varied

from one environment to another. The distribution of physiological properties among these bacteria is discussed and compared with that found in pine forest soil isolates.

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